



## Infrared methodologies for the assessment of skin temperature daily rhythm in two domestic mammalian species

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### ABSTRACT

To assess the accuracy of infrared methodologies for daily rhythm monitoring of skin temperature, five clinically healthy Italian Saddle gelding horses, and five not pregnant and not lactating Camosciata goats, were monitored every 4 h over a 48 h period. The horses were housed in individual boxes, while the goats in two indoor pens, under natural photoperiod and natural environmental temperature. In each animal, skin temperature was recorded with the use of a digital infrared camera and a non-contact infrared thermometer, in five regions: neck, shoulder, ribs, flank and croup. Recorded values were compared with the well-established daily rhythm of rectal temperature. Rectal temperature was recorded at the same time by means of a digital thermometer. In horses, a lower value of skin temperature was recorded using the infrared thermometer for the croup region compared to shoulder and flank; a lower value of skin temperature was recorded using thermography for the croup region compared to the shoulder. In goats, a lower value of skin temperature was recorded using the infrared thermometer for the croup region compared to the flank. In both species, higher values of rectal temperature were observed, compared to the temperature recorded at the skin regions using the other two methodologies. Cosinor rhythmometry showed a daily rhythm of rectal and skin temperature recorded using both methodologies in all the examined regions. General linear model (GLM) showed statistically significant effect of breed on all rhythmic parameters; of day of monitoring on amplitude; of site of recording (rectal vs skin regions) on mesor, amplitude and acrophase; and no effect of methodologies used. The results of this study show the differences in rhythmicity of various body regions temperature and their differences in comparison with daily rhythm rectal temperature. The use of infrared methodologies was inaccurate in assessing body core temperature, but its use could be considered for the evaluation of inflammation in the different body sites.

### 1. Introduction

The homeothermic animal is able to maintain a balance between heat production and heat loss in order to keep its temperature constant (Arfuso et al., 2016). The body produces continuously heat that is dissipated through the surface in several ways (Piccione et al., 2005a). The evaluation of body temperature represents a valuable tool to monitor the physiologic status, the welfare and the stress responses of animals. Temperature monitoring is an important part of clinical medicine that enables early detection of changes in the patient clinical condition associated with infection, systemic inflammatory response syndrome, immune-mediate diseases, neoplasia and shock (Battersby et al., 2006). In mammals, core temperature is homeostatically

regulated at around 37–39 °C despite large variations in the environment temperature. Daily oscillations of up to 2 or 3 °C above and below the thermoregulatory set point area are typical of mammalian physiology (Refinetti and Menaker, 1992), showing an endogenous daily cycle (Refinetti and Piccione, 2003). Measuring body temperature in animals includes the use of rectal thermometers, tympanic infrared thermometers and thermal microchips (Goodwin, 1998). Rectal temperature measurement requires the patient to be restrained. This invasive procedure, in reactive animals, might alter the result of the measurement, therefore compromising its interpretation, and the consequent diagnostic and therapeutic measures. Moreover, this procedure may cause a danger for the operator and alternative ways of temperature measuring could be useful and practical for the clinician. Infrared thermography

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**Table 1**

Mean  $\pm$  sd of rhythmic parameters (Mesor, amplitude, acrophase and robustness of rhythm) of temperature recorded in horses, expressed in their conventional units, with the statistical significances. Capital letter (A) indicates significant differences versus rectal temperature. Symbol (\*) indicates statistical differences versus croup temperature recorded using infrared camera.

Rectal	Mesor (°C)	Amplitude (°C)	Acrophase (hh:mm)	Robustness (%)
Day 1	37.31 $\pm$ 0.21	0.26 $\pm$ 0.08	18:55 $\pm$ 1h 58 min	53.84 $\pm$ 23.87
Day 2	37.07 $\pm$ 0.21	0.22 $\pm$ 0.06	19:20 $\pm$ 1 h 2 min	47.98 $\pm$ 17.22
<b>Infrared camera</b>				
<b>Neck</b>				
Day 1	32.98 $\pm$ 0.58 <sup>A</sup>	0.95 $\pm$ 0.60 <sup>A</sup>	17:52 $\pm$ 57 min*	59.04 $\pm$ 14.85
Day 2	33.00 $\pm$ 0.29 <sup>A*</sup>	0.75 $\pm$ 0.31	16:16 $\pm$ 2h 15 min*	70.20 $\pm$ 18.13
<b>Shoulder</b>				
Day 1	33.24 $\pm$ 0.23 <sup>A*</sup>	0.97 $\pm$ 0.32 <sup>A</sup>	17:05 $\pm$ 28 min <sup>A</sup>	73.28 $\pm$ 14.84
Day 2	33.08 $\pm$ 0.32 <sup>A*</sup>	0.64 $\pm$ 0.32	16:05 $\pm$ 2 h 15 min <sup>A</sup>	59.46 $\pm$ 25.35
<b>Ribs</b>				
Day 1	33.17 $\pm$ 0.25 <sup>A</sup>	1.05 $\pm$ 0.22 <sup>A</sup>	16:55 $\pm$ 20 min <sup>A</sup>	72.20 $\pm$ 16.47
Day 2	32.94 $\pm$ 0.45 <sup>A</sup>	0.61 $\pm$ 0.07	15:30 $\pm$ 2 h 10 min <sup>A</sup>	71.32 $\pm$ 26.51
<b>Flank</b>				
Day 1	32.92 $\pm$ 0.47 <sup>A</sup>	1.13 $\pm$ 0.33 <sup>A</sup>	16:47 $\pm$ 1 h 5 min <sup>A</sup>	77.94 $\pm$ 11.91
Day 2	33.01 $\pm$ 0.52 <sup>A*</sup>	0.64 $\pm$ 0.20	14:58 $\pm$ 2h 38 min <sup>A</sup>	50.18 $\pm$ 17.47
<b>Croup</b>				
Day 1	32.42 $\pm$ 0.47 <sup>A</sup>	1.58 $\pm$ 0.43 <sup>A</sup>	15:52 $\pm$ 40 min <sup>A</sup>	82.26 $\pm$ 18.15
Day 2	32.19 $\pm$ 0.57 <sup>A</sup>	0.98 $\pm$ 0.46	15:45 $\pm$ 2 h 10 min <sup>A</sup>	60.50 $\pm$ 42.48
<b>Infrared thermometer</b>				
<b>Neck</b>				
Day 1	33.01 $\pm$ 0.27 <sup>A</sup>	0.84 $\pm$ 0.26	16:15 $\pm$ 35 min	59.32 $\pm$ 35.84
Day 2	32.19 $\pm$ 0.28 <sup>A</sup>	0.91 $\pm$ 0.21 <sup>A</sup>	17:27 $\pm$ 1 h 5 min	70.56 $\pm$ 19.61
<b>Shoulder</b>				
Day 1	32.92 $\pm$ 0.26 <sup>A</sup>	0.90 $\pm$ 0.38	17:02 $\pm$ 1 h 15 min	59.10 $\pm$ 27.01
Day 2	32.64 $\pm$ 0.44 <sup>A</sup>	0.78 $\pm$ 0.38	16:45 $\pm$ 1 h 50 min	62.70 $\pm$ 29.67
<b>Ribs</b>				
Day 1	32.91 $\pm$ 0.35 <sup>A</sup>	0.91 $\pm$ 0.29	17:21 $\pm$ 1 h 25 min	75.22 $\pm$ 11.81
Day 2	32.34 $\pm$ 0.27 <sup>A</sup>	0.91 $\pm$ 0.15 <sup>A</sup>	17:25 $\pm$ 56 min	77.88 $\pm$ 12.18
<b>Flank</b>				
Day 1	33.03 $\pm$ 0.29 <sup>A</sup>	0.95 $\pm$ 0.26 <sup>A</sup>	17:33 $\pm$ 1 h 38 min	69.08 $\pm$ 13.41
Day 2	32.43 $\pm$ 0.26 <sup>A</sup>	0.90 $\pm$ 0.16 <sup>A</sup>	16:50 $\pm$ 47 min	72.70 $\pm$ 18.24
<b>Croup</b>				
Day 1	32.31 $\pm$ 0.63 <sup>A</sup>	1.25 $\pm$ 0.43 <sup>A</sup>	17:31 $\pm$ 1 h 33 min	78.96 $\pm$ 19.81
Day 2	31.66 $\pm$ 0.32 <sup>A</sup>	1.35 $\pm$ 0.27 <sup>A</sup>	16:32 $\pm$ 37 min	75.08 $\pm$ 18.13

represents a remote, rapid and non-invasive method of measuring temperature. Infrared thermography uses a specialized camera that measures thermal radiation or surface temperature emitted from an object and displays the different temperatures detected as an image, with different colors or a shade of the image representing the different temperatures (Johnson et al., 2011). In human medicine, particularly in pediatric medicine, where patients are often uncooperative, a good alternative is the noncontact infrared thermometer. Fraden and Lackey (1991) reported the differences between different body sites by establishing the relationship between the temperature of ear and the more traditional thermometry sites. Tympanic and esophageal temperatures were equivalent to core body temperature taken from a major artery (pulmonary or carotid), whereas rectal temperature exceeded core temperature or was significantly different from the other body sites. In veterinary medicine, infrared thermometer has been investigated in various species of mammals, such as dog, goats and horses (Piccione et al., 2011b; Ramey et al., 2011; Giannetto et al., 2017). Some studies

**Table 2**

Mean  $\pm$  sd of rhythmic parameters (Mesor, amplitude, acrophase and robustness of rhythm) of temperature recorded in goats, expressed in their conventional units, with the statistical significances. Capital letter (A) indicates statistical differences versus rectal temperature. Symbols indicate statistical differences due to the site of measurement (\* vs croup camera; ● vs flank thermometer).

Rectal	Mesor (°C)	Amplitude (°C)	Acrophase (hh:mm)	Robustness (%)
Day 1	38.47 $\pm$ 0.07	0.41 $\pm$ 0.19	19:45 $\pm$ 40 min	86.52 $\pm$ 9.27
Day 2	38.46 $\pm$ 0.04	0.51 $\pm$ 0.18	20:04 $\pm$ 28 min	86.40 $\pm$ 8.88
<b>Infrared camera</b>				
<b>Neck</b>				
Day 1	32.50 $\pm$ 0.96 <sup>A</sup>	1.94 $\pm$ 0.51	15:10 $\pm$ 55 min <sup>A</sup>	88.88 $\pm$ 6.14
Day 2	32.62 $\pm$ 0.78 <sup>A</sup>	2.15 $\pm$ 0.37	16:30 $\pm$ 18 min <sup>A</sup>	80.38 $\pm$ 7.53
<b>Shoulder</b>				
Day 1	32.62 $\pm$ 1.08 <sup>A</sup>	1.97 $\pm$ 0.46	14:55 $\pm$ 45 min <sup>A</sup>	87.68 $\pm$ 11.45
Day 2	32.79 $\pm$ 1.03 <sup>A</sup>	2.09 $\pm$ 0.48	16:20 $\pm$ 30 min <sup>A</sup>	84.62 $\pm$ 6.44
<b>Ribs</b>				
Day 1	32.73 $\pm$ 1.14 <sup>A</sup>	1.88 $\pm$ 0.43*	15:00 $\pm$ 24 min <sup>A</sup>	87.04 $\pm$ 9.25
Day 2	32.93 $\pm$ 1.14 <sup>A</sup>	1.49 $\pm$ 0.32*	16:18 $\pm$ 54 min <sup>A</sup>	82.36 $\pm$ 4.05
<b>Flank</b>				
Day 1	32.63 $\pm$ 1.24 <sup>A</sup>	2.24 $\pm$ 0.68	14:52 $\pm$ 30 min <sup>A</sup>	82.22 $\pm$ 21.32
Day 2	33.16 $\pm$ 1.07 <sup>A</sup>	1.65 $\pm$ 0.27	15:23 $\pm$ 35 min <sup>A</sup>	83.35 $\pm$ 6.77
<b>Croup</b>				
Day 1	31.89 $\pm$ 0.86 <sup>A</sup>	2.53 $\pm$ 0.49	15:12 $\pm$ 32 min <sup>A</sup>	85.94 $\pm$ 8.43
Day 2	32.46 $\pm$ 0.55 <sup>A</sup>	2.36 $\pm$ 0.55	15:26 $\pm$ 35 min <sup>A</sup>	82.86 $\pm$ 4.84
<b>Infrared thermometer</b>				
<b>Neck</b>				
Day 1	32.33 $\pm$ 0.94 <sup>A</sup>	1.80 $\pm$ 0.65●	15:26 $\pm$ 57 min <sup>A</sup>	71.26 $\pm$ 27.49
Day 2	32.51 $\pm$ 0.49 <sup>A</sup>	1.99 $\pm$ 0.24●	16:35 $\pm$ 35 min <sup>A</sup>	83.40 $\pm$ 5.60
<b>Shoulder</b>				
Day 1	32.99 $\pm$ 1.00 <sup>A</sup>	1.20 $\pm$ 0.66	16:26 $\pm$ 1 h 16 min <sup>A</sup>	70.56 $\pm$ 22.24
Day 2	33.18 $\pm$ 0.71 <sup>A</sup>	1.67 $\pm$ 0.40	16:16 $\pm$ 1h 40 min <sup>A</sup>	78.94 $\pm$ 14.26
<b>Ribs</b>				
Day 1	32.94 $\pm$ 1.03 <sup>A</sup>	1.38 $\pm$ 0.25	16:28 $\pm$ 1 h 4 min <sup>A</sup>	66.40 $\pm$ 28.86
Day 2	33.16 $\pm$ 0.88 <sup>A</sup>	1.37 $\pm$ 0.55	17:14 $\pm$ 3h <sup>A</sup>	66.92 $\pm$ 25.65
<b>Flank</b>				
Day 1	33.83 $\pm$ 0.85 <sup>A</sup>	1.23 $\pm$ 0.72	16:18 $\pm$ 1 h 15 min <sup>A</sup>	72.23 $\pm$ 26.03
Day 2	33.28 $\pm$ 1.26 <sup>A</sup>	1.07 $\pm$ 0.52	16:09 $\pm$ 1h 30min <sup>A</sup>	66.58 $\pm$ 17.23
<b>Croup</b>				
Day 1	32.30 $\pm$ 1.00 <sup>A</sup>	2.09 $\pm$ 0.77●	15:56 $\pm$ 1 h 23 min <sup>A</sup>	65.05 $\pm$ 27.50
Day 2	32.83 $\pm$ 0.38 <sup>A</sup>	1.93 $\pm$ 0.45●	15:50 $\pm$ 1h 46 min <sup>A</sup>	75.62 $\pm$ 16.76

on horses reported a higher muscle temperature than rectal temperature (Lindholm and Saltin, 1974; Carlson, 1983) and a higher rectal temperature than central venous temperature and skin temperature (Morgan, 1997). In particular, higher rectal temperature than auricular temperature was observed both in a light/dark cycle and during constant darkness (Piccione et al., 2011). It has also been demonstrated (Aschoff and Heiss, 1972) that heat loss shows diurnal fluctuations determined by conductance, evaporation, convection and radiation. Changes in heat loss via convection and radiation are primarily caused by variations in skin blood flow, with consequent changes in skin temperature. In veterinary medicine, another aspect to take into consideration is the presences of hair and, in particular, of fleece in small ruminants. In sheep, considerable difficulties in the application of infrared thermometry were reported (Katsoulos et al., 2016). As for other physiological variables (Piccione et al., 2005b), daily rhythmicity of body temperature was observed in goats housed under two different ambient temperature; when the animals were kept under high

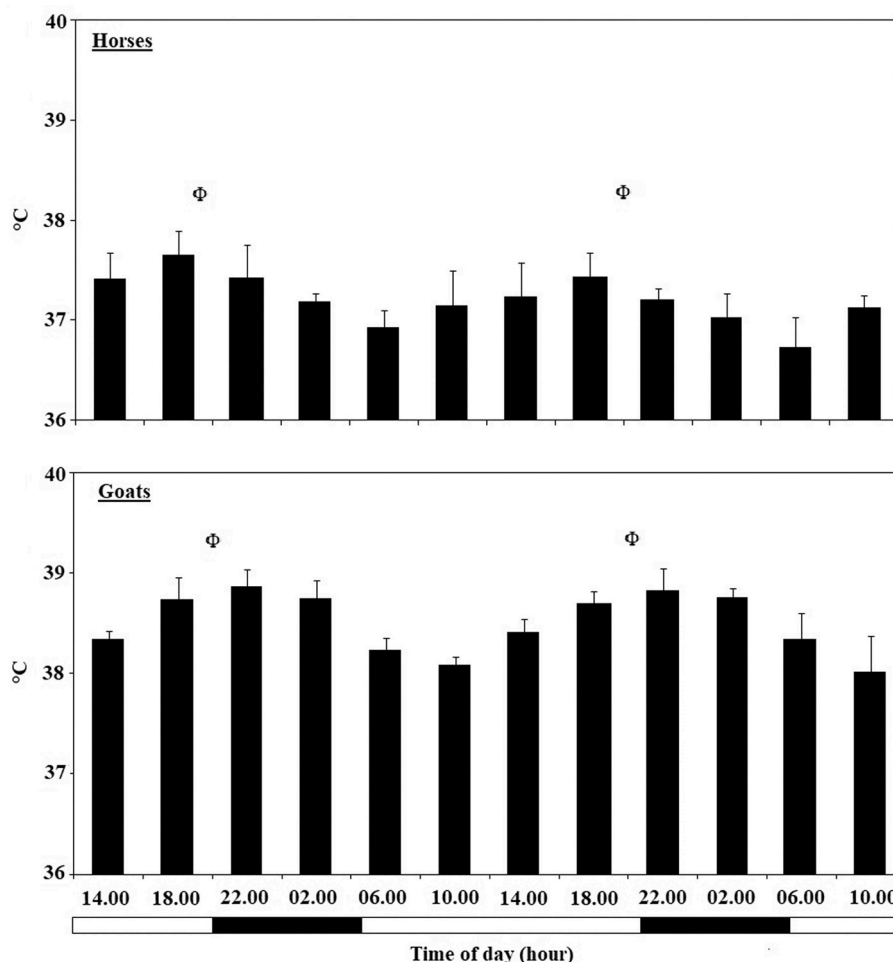


Fig. 1. Daily fluctuation of rectal temperature recorded in horses and goats during the study period. Black and white bars indicate the scoto-e photo-phase of experimental photoperiod.

environmental temperature, the body temperature remained relative stable despite wide range of environmental temperature excursions, whereas the auricular temperature (AT) increased (Piccione et al., 2005c). A study conducted under different external temperature showed the accuracy of the thermoregulatory system in goats (Piccione and Refinetti, 2003). Comparing a large number of studies conducted on different species, it was showed that the amplitude of the daily rhythm of body temperature is different (Aschoff, 1982; Refinetti and Menaker, 1992). The differences were due to the level of physical restraint of the subjects, the thermal sensors location within the body, the temporal spacing of successive measurements and to the averaging of separate measurements or separate subjects. A study of 11 different mammals' species using exactly the same equipment and housing conditions indicated that the studied species had clearly different mean amplitudes of body temperature daily rhythm (Refinetti, 1999). On the basis of this knowledge, the aim of this study was to establish the accuracy of skin temperature assessment as an alternative method to rectal temperature in veterinary medicine. In this regard, we compared the rectal temperature rhythmicity with the various skin body regions temperature rhythmicity detected using two different infrared methodologies in large and small domestic animals.

## 2. Materials and methods

### 2.1. Animal and experimental design

The study was carried out in Sicily, Italy (38°00'49"N 15°25'18"E,

80 m above sea level). Five Italian Saddle gelding horses (10–11 years old, with a mean body weight of  $510 \pm 27$  kg) and five not pregnant and not lactating Camosciata goats (2–4 years old, with a mean body weight of  $37 \pm 2$  kg) were enrolled. Before the beginning of the study, the health status was evaluated based on rectal temperature, heart rate, respiratory rate, appetite, faecal consistency and hematologic profile. All the enrolled animals were clinically healthy and free from internal and external parasites. For the entire study period, each horse was housed in individual boxes ( $3.20 \times 3.20$  m), equipped with an opening windows; goats were kept in two indoor pens. All animals were exposed to a natural photoperiod (sunrise at 5:20 h, sunset at 20:27 h over the study period) and natural environmental temperature. Thermal and hygrometric records were carried out inside the box and pen for the whole study by means of a data logger (Gemini, UK), and they followed the normal seasonal pattern for the location (mean ambient temperature and mean relative humidity of 21 °C and 55%, respectively). The temperature-humidity index (THI) was 66.36 °C. The THI value, an indicator of thermal comfort for goat, was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for ruminant species (Potter and Jacobsen, 2000):

$$\text{THI (}^{\circ}\text{C)} = T^{\circ}\text{ambient} + (0.36 \times \text{point of steam condensation}) + 41.5$$

The horses were fed three times a day (06.30, 12.00, and 19.00) with good-quality hay and concentrate. This feeding schedule has been previously shown not to affect the body temperature rhythm of the horse (Piccione et al., 2002). All goats were fed with alfalfa (*Medicago sativa* L.) hay and 250g/animal of concentrate distributed twice a day. For both

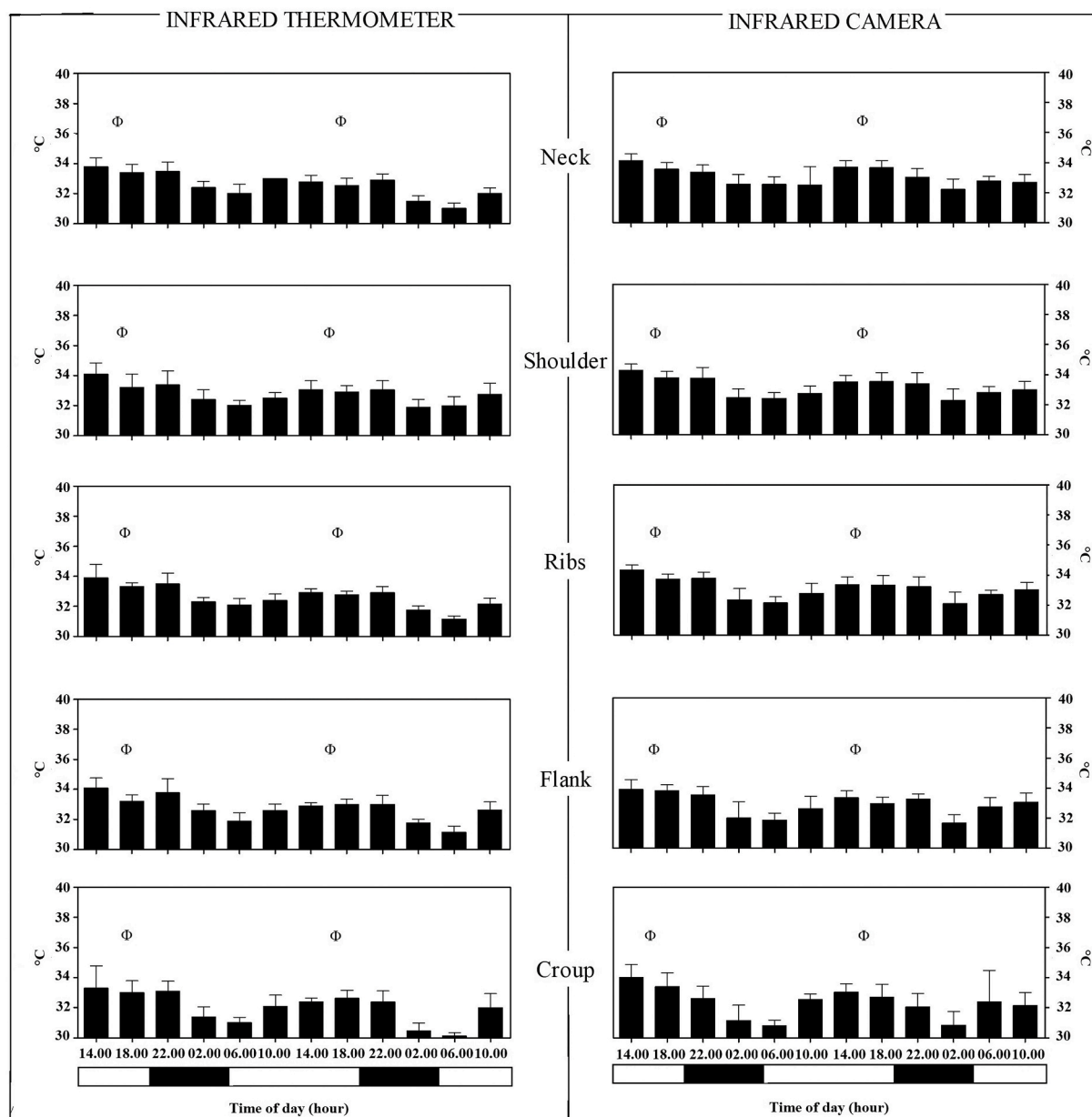


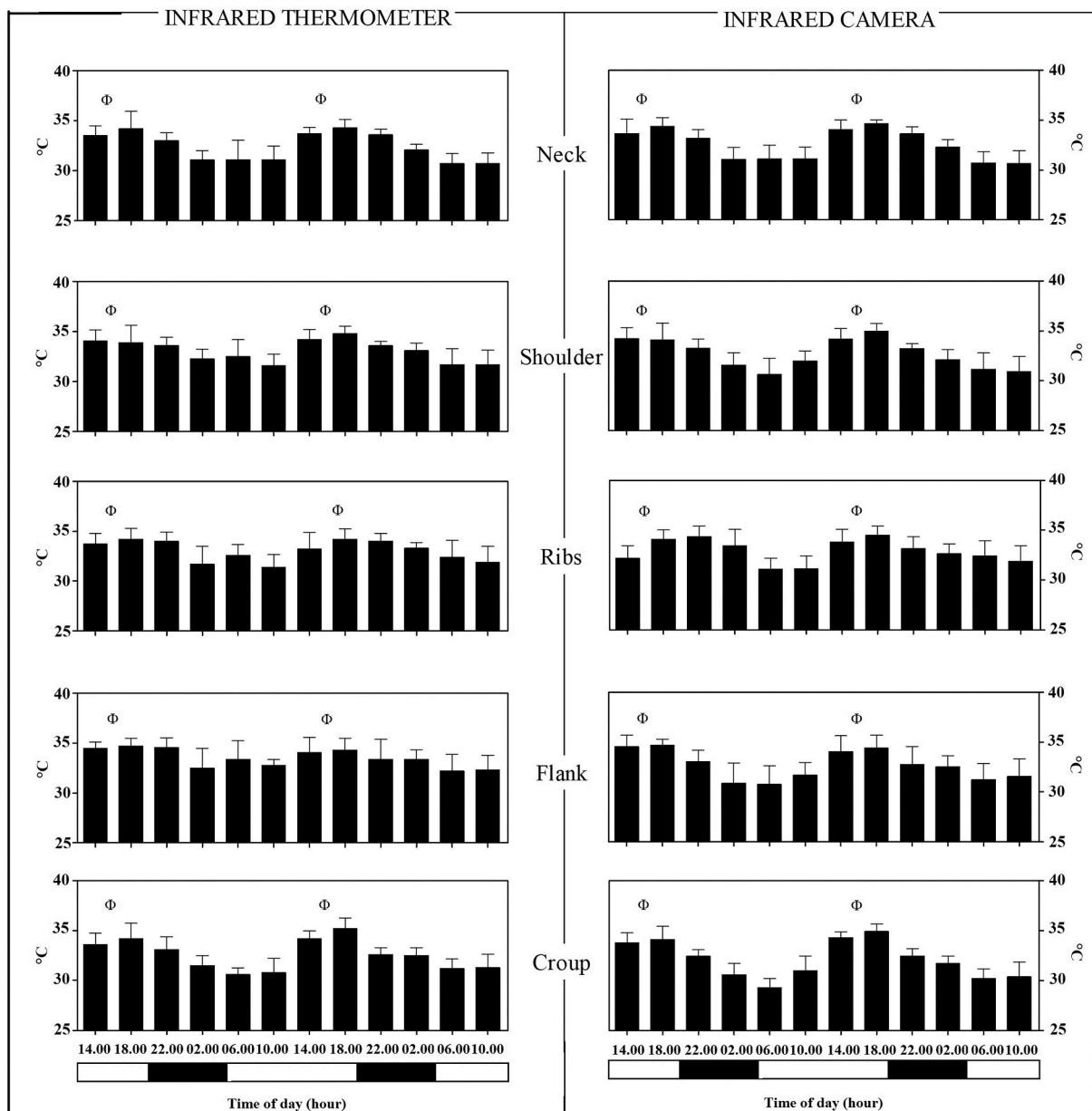
Fig. 2. Daily fluctuation of skin temperature recorded in the five regions (neck, shoulder, ribs, flank and croup) in horses during the two days of monitoring. Black and white bars indicate the scoto-e photo-phase of experimental photoperiod.

species water was available ad libitum.

## 2.2. Temperature assessment

Rectal and skin temperature were recorded every 4 h for a 48 h period starting at 14:00 on day 1 and ending at 10:00 on day 3 of monitoring. Rectal temperature was recorded with a digital thermometer (model HI92704, Hanna Instruments), with resolution of 0.1 °C, that was inserted 15 cm and 9 cm into the rectum, in horses and goats respectively. Two methods were used to record cutaneous temperature in five regions of interest: neck, shoulder, ribs, flank and croup. Thermal images of the animals were taken using a digital infrared camera (ThermaCam P25 Model, Flir Systems, Boston, MA, USA). Absolute mean temperature of each region of interest was obtained using thermography software (Thermacam Researcher Basic 2.8 software, FLIR, Wilsonville, Oregon, USA). To reduce the effects of environmental factors on thermographic readings, all images were scanned at 1 m from the

subject. The settings of the camera were as follows: range of temperature 20–40 °C; emissivity of skin: 0.97; reflected air temperature (Trifl): 20 °C; distance between camera and body surface (Dist): 1 m; field of view (FOV): 23°. The detector consisted of a focal plane array (FPA) uncoiled microbolometer with the following specifications: 320 × 240 pixels resolution, thermal sensitivity of 0.08 °C (at 30 °C), spatial resolution (IFOV) of 1.3 mrad, spectral range between 7.5 and 13 μm accuracy ±2 °C. Automatic corrections based on user input were conducted for reflected ambient temperature, distance, relative humidity and atmospheric transmission. Moreover, in each region of interest, cutaneous temperature was measured by means of a non-contact infrared thermometer (Infrared Thermometer Model THM010-VT001, Mediad Inc., Cerritos, CA, US) placed at the distance of 5 cm from the measurement site, with the following specifications: accuracy ±0.3 °C, range of temperature from 0 °C to 60 °C, thermal sensitivity 0.2 °C. All animals were cooperative to all recording. All housing and care conformed to the standard recommended by the Guide for the Care and Use of Laboratory



**Fig. 3.** Daily fluctuation of skin temperature recorded in the five regions (neck, shoulder, ribs, flank and croup) in goats during the two days of monitoring. Black and white bars indicate the scoto-e photo-phase of experimental photoperiod.

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### 2.3. Statistical analysis

The obtained data were expressed as mean  $\pm$  standard deviation (SD). Data were normally distributed ( $p > 0.05$ , Kolmogorov-Smirnov test). We applied a trigonometric statistical model to each animal values at each time series, in order to analytically describe the periodic phenomenon, characterizing the main rhythmic parameters according to the single cosinor procedure (Nelson et al., 1979). Four rhythmic parameters were determined: mesor, amplitude (the difference between the peak, or trough, and the mean value of a wave), acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). A multivariate for repeated measures, General Linear Model (GLM), was applied on temperature values to establish the effect of breed, day of monitoring, time of day, site of recording and methodologies used. Multivariate GLM was applied on the rhythmic

parameters to investigate statistical differences due to breed, day of monitoring, site of recording and methodologies used. Bonferroni's test was applied for post hoc comparison.  $P < 0.05$  was considered statistically significant. Statistical analysis was performed using the STATISTICA software package (STATISTICA 7 Stat Software Inc., Tulsa, Oklahoma).

### 3. Results

The application of cosinor method showed a daily rhythm of rectal temperature and skin temperature recorded using both methodologies in all examined skin regions (Tables 1 and 2).

The application of GLM showed no significant effect of monitoring day ( $p = 0.10$ ;  $F = 2.61$ ;  $df = 1$ ) and methodologies used ( $p = 0.70$ ;  $F = 0.14$ ;  $df = 1$ ), but found statistical significant effect of species ( $p = 0.02$ ;  $F = 4.96$ ;  $df = 1$ ), time of the day ( $p = 0.0001$ ;  $F = 136.56$ ;  $df = 5$ ) and site of recording ( $p = 0.0001$ ;  $F = 295.95$ ;  $df = 5$ ). In particular, in horses

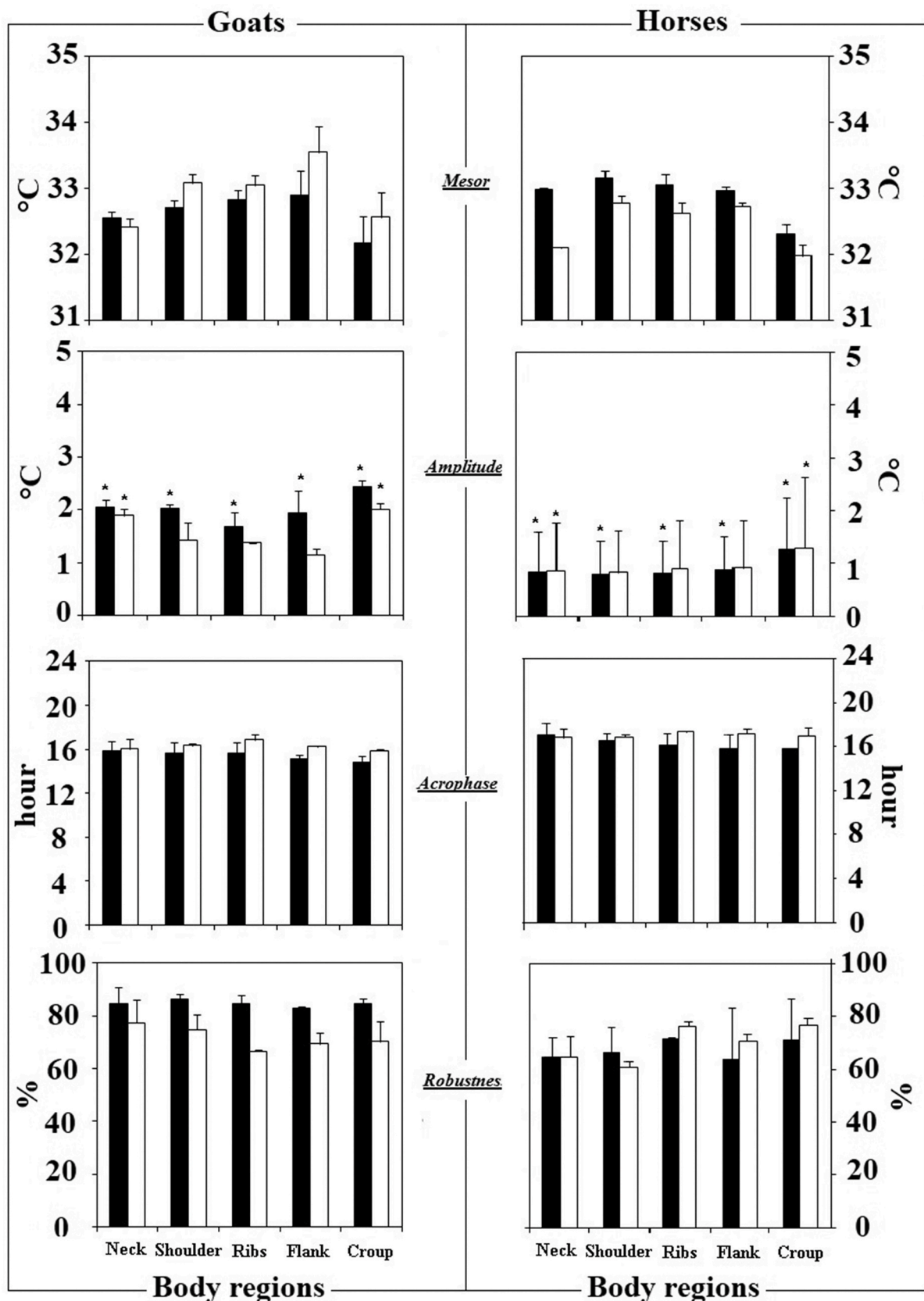


Fig. 4. Circadian parameters (Mesor, amplitude, acrophase and robustness), expressed in their conventional unit, of skin temperature recorded in goats and horses using infrared thermocamera (■) and infrared thermometer (□), with statistical differences. Significance: \*vs other species  $p < 0.001$ .



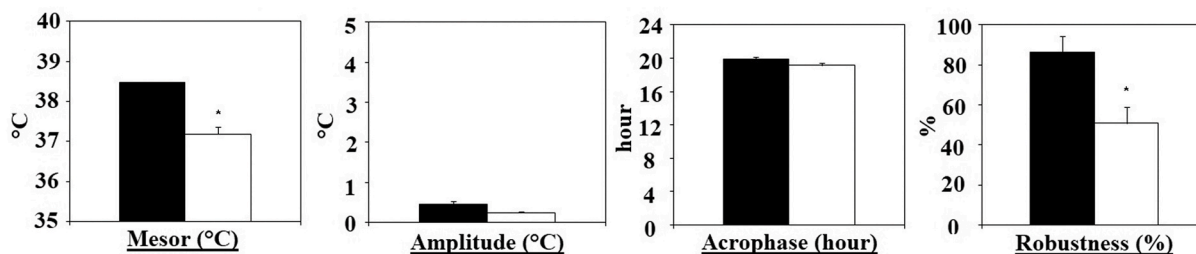


Fig. 5. Circadian parameters (Mesor, amplitude, acrophase and robustness), expressed in their conventional unit, of rectal temperature recorded in goats (■) and horses (□), with statistical differences. Significance: \*vs goats  $p < 0.001$ .

a lower value of skin temperature was recorded using the infrared thermometer at the croup region compared to shoulder and flank; a lower value of skin temperature was recorded using thermography at the croup region compared to the shoulder. In goats, a lower value of skin temperature was recorded using the infrared thermometer at the croup region compared to the flank. A higher value of rectal temperature was observed compared to the skin regions temperature recorded using both methodologies, in both species. Figs. 1–3 show the daily fluctuation of temperatures recorded in the different species. The application of GLM on the rhythmic parameters showed statistical significant effect of breed on all rhythmic parameters ( $df = 1$ ; Mesor:  $p = 0.05$ ;  $F = 3.65$ ; Amplitude:  $p = 0.001$ ;  $F = 176.36$ ; Acrophase:  $p = 0.007$ ;  $F = 10.16$ ; Robustness:  $p = 0.0001$ ;  $F = 18.77$ ); of day of monitoring on amplitude ( $p = 0.05$ ;  $F = 3.98$ ;  $df = 1$ ), of site of recording on mesor ( $p = 0.0001$ ;  $F = 104.30$ ;  $df = 5$ ), amplitude ( $p = 0.0001$ ;  $F = 26.41$ ;  $df = 5$ ) and acrophase ( $p = 0.0001$ ;  $F = 21.21$ ;  $df = 5$ ); and no effect of methodologies used. Bonferroni post hoc comparison results are reported in Tables 1 and 2. The mean between the two day of monitoring did not show statistical differences. The mean ( $\pm$ SD) of the two day of monitoring are reported in Figs. 4 and 5 together with the statistical differences due to species.

#### 4. Discussion

The results of this study indicate a robust daily rhythm of rectal temperature in horses and goats. These results are in accordance with previous findings establishing that body temperature rhythmicity, measured by means of rectal thermometer, reaches its acrophase in scotophase, exhibiting high robustness (Piccione et al., 2005a; Refinetti and Piccione, 2005), and they were within the range reported for goats. However, previous studies conducted in goats showed that different acrophase values were obtained ranging from 9 to 16 h after sunrise (Piccione and Refinetti, 2003). Also, these results allowed to use the measurement of rectal temperature, whose oscillation is a well-established marker of the circadian clock operation (Refinetti, 2010), as control of skin temperature daily rhythm.

Skin temperature was lower than rectal temperature of about 4.50° and 5.00 °C, in horses and goats respectively. Statistical differences of temperature measurements were also recorded between body regions in the same species. In particular, temperature values using the infrared thermometer were lower at level of the croup region respect the flank, both in horses and goats. Moreover, in horses, this difference was observed compared to the shoulder and it was confirmed by the use of both infrared methodologies. The differences in the value of the measured temperature are probably due to the thinner fur and muscle mass of these regions (Rizzo et al., 2017).

Infrared methodologies determine the surface body temperature by measuring the natural electromagnetic radiation emitted from the body with good performance at various body sites. However, the obtained values are not always well correlated with rectal temperature (Katsoulou et al., 2016). A previous study showed that skin temperature, recorded at abdomen and neck levels, reflects the oscillation of rectal temperature (Piccione et al., 2005a). The same cannot be said for our findings. The

daily rhythm of rectal temperature showed its acrophase about 14 h after sunrise, in both studied species, in horses with an anticipation of about 4 h if the skin temperature was recorded with thermocamera, and about 3 h if temperature was recorded with infrared thermometer. Similar conditions were observed in goats, with an anticipation of about 4 and 5 h, respectively. The reason of the different peak of temperature recorded using the two different methodologies is not clear and needs further investigations. In infant, the daily skin temperature rhythm acrophases recorded at two different sites were much more variable than the daily rectal temperature rhythm and up to 8 h later (Bogh et al., 1994). Even though the daily rhythm of skin temperature showed a good percentage of robustness, this was not statistically different from the percentage of robustness of rectal temperature. Skin temperature daily rhythm showed an instability of the amplitude between the two days of monitoring, with statistical differences between the body regions.

In conclusion, the results of this study showed that body regions present a daily rhythm of skin temperature, with a diurnal acrophase and a high percentage of robustness. The assessed regions showed different Mesor and amplitude values, also in relation to the infrared methodology used.

Based on the results of this study, skin temperature does not seem to be a reliable alternative to rectal temperature recording for body temperature measurement, in both studied species. However, the knowledge of skin temperature daily rhythmicity in various body regions could represent an added value in the diagnosis of inflammatory processes involving the body surface.

#### Author contributions

C.G. designed, performed experiments, analyzed data and wrote the paper, A.F. designed, performed experiments, analyzed data and wrote the paper; E.G. performed experiments and analyzed data; M.G. performed experiments and analyzed data. F.F. performed experiments and analyzed data M.P. performed experiments and analyzed data; G.P. designed, analyzed data and wrote the paper.

#### Declaration of competing interest

The authors declare that they do not have conflict of interest with the data presented in the manuscript.

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